

Peritoneal Fluid from a Febrile Foal

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**What Is Your
Diagnosis?**

Case Presentation

A 3-month-old Quarter Horse filly was presented to the Washington State University College of Veterinary Medicine with a history of intermittent pyrexia of 1-week duration. During this period, the owners noted that the foal appeared hunched over and reluctant to move. Fever (104°F), neutrophilia (11,500 cells/ μ L, reference interval 2700-6700 cells/ μ L), and hyperfibrinogenemia (1000 mg/dL, reference interval 100-800 mg/dL¹) were reported by the referring veterinarian. The foal had received intermittent treatments of flunixin meglumine paste during the last week and had been given oral trimethoprim/sulfamethoxazole the day before and the day of presentation. She had been dewormed at 30 and 44 days of age.

On presentation, the foal was in good body condition. Body temperature was 102°F, heart rate was 88 beats/min, and respiratory rate was 20 breaths/min. Mucous membranes were pink and moist. The foal was mildly dehydrated, and decreased gut sounds were heard. Slight expiratory respiratory effort was noted. Auscultation of the lungs revealed no abnormalities except for mildly harsh lung sounds in the ventral lung fields when a rebreathing bag was used.

Abnormal findings on a CBC and chemistry profile included mature neutrophilia (17,794 cells/ μ L, reference interval 3920-10,350 cells/ μ L¹), monocytosis (868 cells/ μ L, reference interval 120-760 cells/ μ L¹), thrombocytosis (485,000 cells/ μ L, reference interval 200,000-376,000 cells/ μ L¹), hyperfibrinogenemia (1300 mg/dL, reference interval 100-800 mg/dL¹), hypoalbuminemia (1.9 g/dL, reference interval 2.8-3.5 g/dL¹), and hyponatremia (137 mEq/L, reference interval 140-156 mEq/L¹).

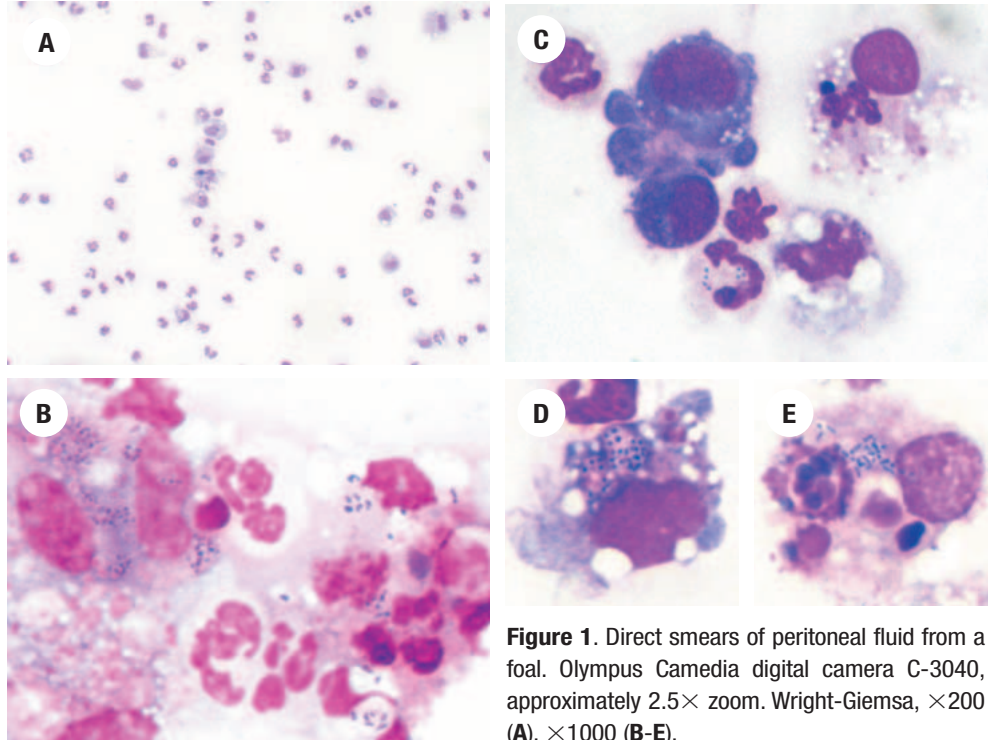


Figure 1. Direct smears of peritoneal fluid from a foal. Olympus Camedia digital camera C-3040, approximately 2.5 \times zoom. Wright-Giemsa, \times 200 (A), \times 1000 (B-E).

Radiography of the thorax was performed, and early peribronchial infiltrates were seen in the cranioventral and caudodorsal lung fields. Abdominal ultrasonography also was performed, and free peritoneal fluid and a large fluid-filled well-circumscribed mass in the left ventral quadrant were seen. Abdominocentesis yielded 2 ml of cloudy yellow fluid. The fluid was submitted for cytologic examination (Figure 1). The fluid had a total nucleated cell count of 115,000 cells/ μ L and a protein concentration of 4.4 g/dL.

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Cytologic Interpretation

Direct smears of the peritoneal fluid were of high cellularity with a proteinaceous background (Figure 1A). The nucleated cell differential count was approximately 78% neutrophils, 20% macrophages, and 2% lymphocytes. The majority of neutrophils were nondegenerate to mildly degenerate. Occasionally, pyknotic neutrophils were seen. Macrophages often contained phagocytized neutrophils (Figure 1, C and E). A population of bacteria was present within both neutrophils (Figure 1, B and C) and macrophages (Figure 1, B, D, and E). Bacterial shape ranged from cocci to short rods. The organisms were arranged individually, in pairs, in short chains, and in loose clusters. A thin, clear area surrounded the majority of organisms. Occasional reactive mesothelial cells also were seen. The cytologic interpretation was septic exudate with mixed (primarily suppurative) inflammation.

Additional Test Results

A Gram's stain of the fluid revealed rare gram-positive cocci with possible gram-negative rods. Polymerase chain reaction (PCR) assay performed on the peritoneal fluid for *Rhodococcus equi* was positive for the VapA plasmid. Aerobic bacterial culture of the peritoneal fluid resulted in heavy growth of *R equi*. There was no anaerobic bacterial growth.

The foal was treated for 7 months with azithromycin and rifampin (initial doses were 10 mg/kg once daily and 5 mg/kg twice daily, respectively). After 4 months of treatment, neutrophilic peritonitis remained, with a total nucleated cell count of 25,100 cells/ μ L, a protein concentration of 3.3 g/dL, and no bacteria seen. Follow-up paracentesis was not performed. One month after cessation of antibiotics, mild neutrophilia (8630 cells/ μ L, reference interval 2700-6700 cells/ μ L) and hyperfibrinogenemia (500 mg/dL, reference interval 100-400 mg/dL) were present. Follow-up ultrasonography was not performed to assess resolution of the abscess. At the time of publication, the foal was clinically healthy except for occasional mild bouts of colic.

Discussion

R equi is a facultative intracellular gram-positive coccobacillus that is a common cause of pneumonia in foals. The organism can also cause multifocal ulcerative colitis and typhlitis with mesenteric lymphadenitis and abdominal abscesses. Arthritis, septic pharyngitis, osteomyelitis, subcutaneous abscesses, uveitis, and abscessation of abdominal organs are less commonly seen.² *R equi* also is associated with infections of immunocompromised³⁻⁵ and rarely immunocompetent⁶ human beings.

Isolation of *R equi* has been reported in many other species, including pigs, buffalo, sheep, deer, koalas, seals, marmosets, alligators, crocodiles, cattle,⁷ dogs,^{6,7} cats,^{6,8} goats,^{7,10-13} and llamas.¹⁴

R equi is primarily a soil organism, and it replicates well in herbivore manure at warm temperatures. The primary route of infection is probably inhalation of infected dust.⁷ Ingestion of organisms is another possible route of infection. Virulence of the organism is correlated with its ability to replicate within macrophages, thought to be associated with inhibition of phagosome/lysosome fusion, and the presence of a large plasmid that results in expression of VapA, a plasmid-encoded surface-expressed lipoprotein of undetermined function.¹⁵ Early diagnosis of *R equi* infection is important because most routinely-used antibiotics do not cure the animal of disease. Lipophilic antimicrobial drugs that can penetrate cells, such as the macrolides and rifampin, are necessary for successful treatment of *R equi* infections but are not used routinely for most bacterial agents.^{2,16}

The most common laboratory abnormalities associated with *R equi* infection in foals are hyperfibrinogenemia and neutrophilic leukocytosis, with or without monocytosis.² Thrombocytosis was reported as a common finding in horses in Ireland.¹⁷ Bacterial culture is the traditional method of diagnosing *R equi* infection. *R equi* readily grows under aerobic conditions on nonselective media routinely used in clinical microbiology laboratories but does not typically grow on MacConkey's agar. Although irregularly round, smooth, mucoid colonies may be seen after 48 hours, culture can take up to 7 days and results may be negative, especially if there has been prior antibiotic administration or if multiple pathogenic bacterial species are present. A commercial kit to aid in the identification of *R equi* isolates is available.⁷ Serologic diagnosis of *R equi* is unreliable.²

More recently, a PCR assay has been developed for the rapid identification of *R equi*. Primers for the 16S ribosomal RNA gene (present in both virulent and avirulent strains of *R equi*) and the *R equi* VapA virulence plasmid (VP) are used for rapid and specific detection of *R equi* and to differentiate between virulent and avirulent strains.¹⁶ PCR performed on tracheal wash fluid was more sensitive and specific for diagnosis of *R equi* pneumonia than were the other available diagnostic tests.¹⁸ Using clinical diagnosis as the final reference standard, VP PCR had a diagnostic sensitivity of 100% and specificity of 90.6%, in contrast to the sensitivity and specificity of standard microbial culture of tracheal wash fluid (57.1% and 93.8%, respectively) and serology (62.5% and 75.9%, respectively). For clinical use, PCR typically is performed using primers for only the VapA plasmid.

R equi is most commonly associated with pneumonia in foals, but enteritis, lymphadenitis, and peritonitis may be seen concurrently, or even without respiratory signs. The affected foal of this report presented with nonspecific clinical signs and had peritonitis and a large abdominal abscess. Subsequent evaluation of the respiratory system was normal; the mild respiratory changes seen at presentation were most likely due to pyrexia and recent transportation. All of the most common laboratory abnormalities associated with *R equi* infection, ie, hyperfibrinogenemia, neutrophilia, monocytosis, and thrombocytosis, were present in this foal. Cytologic examination of the peritoneal fluid revealed high numbers of bacteria within macrophages and neutrophils. Although macrophages can phagocytize bacteria, neutrophils are the predominant phagocytic cell seen in most bacterial infections. It is possible that a high number of bacteria within macrophages is an indication of *Rhodococcus sepsis*. The pleomorphic organisms in this case were consistent with *R equi* infection. The significance of the thin clear area surrounding the organisms is unknown. Also unknown is whether the clear areas are typical of the organism, since there are few published cytologic descriptions of *R equi*.

In a previous report of 3 foals with *R equi* enteritis, colonic lymphadenitis, and peritonitis with nonrespon-

sive bronchopneumonia, all foals were euthanized after unsuccessful therapy.¹⁹ Early diagnosis and treatment of *R equi* infections are considered important for the successful management of cases. Although VP PCR should not be used without standard culture because of the high probability of multiple bacterial pathogens, it is a more sensitive and specific test for pathogenic *R equi* and can provide results more quickly. The test is commercially available for veterinarians through the Washington Animal Disease Diagnostic Laboratory (Washington State University, College Station, Pullman, Wash, USA) and the Clinical Microbiology Laboratory at North Carolina State University (College of Veterinary Medicine, Raleigh, NC, USA). The presence of characteristic organisms in a cytologic sample can alert clinicians to the probability of *R equi* infection so that efforts can be directed in a timely manner towards proper therapeutic and diagnostic approaches. ◇

Key Words: Foal, PCR, peritoneal fluid, *Rhodococcus equi*, septic exudate

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